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Contributions of Neural Tone to *In Vivo* Passive Muscle–Tendon Unit Biomechanical Properties in a Rat Rotator Cuff Animal Model

SANDEEP MANNAVA,[1,2] WALTER F. WIGGINS,[1,2] KATHERINE R. SAUL,[3,4] JOEL D. STITZEL,[3,4] BETH P. SMITH,[1] L. ANDREW KOMAN,[1] THOMAS L. SMITH,[1] and CHRISTOPHER J. TUOHY[1]

[1]Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1070, USA; [2]The Neuroscience Program, Wake Forest University Graduate School of Arts and Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1070, USA; [3]Department of Biomedical Engineering, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1022, USA; and [4]VT-WFU School of Biomedical Engineering and Sciences, Medical Center Boulevard, Winston-Salem, NC 27157-1022, USA

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Abstract—Passive viscoelastic properties of muscle–tendon units are key determinants of intra- and post-operative success. Atrophied, retracted, and stiff muscle–tendon units are technically challenging to manipulate and perform poorly after surgical repair. This study employs botulinum neurotoxin A (BoNT-A)-mediated inhibition of presynaptic acetylcholine release to examine *in vivo* neural contributions to soft-tissue biomechanical properties. *In vivo* load-relaxation and active muscle contractile force testing protocols were performed in the rat rotator cuff model. The passive properties were assessed using linear regression analysis and Fung's quasi-linear viscoelastic (QLV) model. BoNT-A injected muscle–tendon units had a significant reduction in force of contraction ($p = 0.001$). When compared to saline injected controls, the BoNT-A significantly decreased parameter 'A' of the QLV model, which represents the linear elastic response ($p = 0.032$). The viscous properties in the BoNT-A treatment group were not significantly different from saline injected controls, as determined by comparison of QLV model parameters 'C,' 'τ_1,' and 'τ_2.' In conclusion, neural tone contributes significantly to muscle–tendon unit passive biomechanical properties. Pre-surgical treatment with BoNT-A may improve the rehabilitation of muscle by altering its passive elastic properties. Accordingly, pharmacological modulation of skeletal muscle stiffness with BoNT-A increases flexibility, potentially improving function. Chemical denervation with BoNT-A may also improve the manipulation of stiff and difficult to mobilize muscles during surgical procedures.

Keywords—Botulinum neurotoxin A, Soft-tissue biomechanics, Rat rotator cuff, Skeletal muscle tone.

Address correspondence to Sandeep Mannava, Department of Orthopaedic Surgery, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1070, USA. Electronic mail: smannava@wfubmc.edu

INTRODUCTION

Approximately 300,000 rotator cuff repair surgeries are performed annually in the United States, as rotator cuff tears are a common cause of upper extremity pain and disability.[1] Chronically torn rotator cuffs can pose an operative challenge because the scarred and retracted muscle–tendon units are difficult to manipulate surgically.[17,18,39] Studies in rat,[14,15] sheep,[6,13] dog,[39] and human rotator cuff[7] have demonstrated that the muscle–tendon unit becomes retracted and stiffer after injury, which is attributed to changes in the muscle's structure.[6,13–15,39] Specifically, as the muscle atrophies following tendon injury, it undergoes sarcopenia,[45] decreased muscle volume,[2,29,39] fibrofatty infiltration,[2,29,30,39] decreased capillary density,[6,13,20,39] and muscle fiber-type switching.[2]

In some instances, full excursion of the injured muscle–tendon unit back to its anatomical footprint on the humeral head is not possible during surgical repair of torn rotator cuffs.[7,15] Even when operative repair is possible, the repair tension might be excessive, leading to early repair site failure or re-tears after surgery.[15,32,33] Other investigators have demonstrated that repair tension, which is determined by the muscle–tendon unit biomechanical properties, is an important factor that influences tendon-to-bone healing in the rotator cuff.[15] In particular, excessive repair tension has been associated with impaired tendon-to-bone healing[15] and worse patient-perceived functional outcomes after rotator cuff repair surgery.[7]

Passive skeletal muscle–tendon unit load-relaxation properties are key determinants of operative success

following tendon repair surgery.[11,15] Most biomechanical studies occur in the *ex vivo* or *in vitro* setting and focus on structural components of the muscle, such as actin, myosin, titin, and desmin.[11] However, these studies fail to evaluate the influence of the nervous system on skeletal muscle tone.[11] Surgical and rehabilitation experience with patients who have sustained neurological insults, such as cerebral palsy or cerebrovascular accidents, demonstrates that the nervous system influences the passive biomechanical properties of muscle.[3,22,36,40]

The purpose of the current study is to use botulinum neurotoxin A (BoNT-A) to examine and quantify *in vivo* neurological contributions to skeletal muscle tone, which consists of elasticity and viscosity. BoNT-A is a potent, naturally occurring toxin that results in a temporary and reversible muscle paresis through pre-synaptic neurological blockade of acetylcholine release.[24,36,40] The present study employs Fung's quasi-linear viscoelastic (QLV) model to characterize the load-relaxation behavior of muscle–tendon units in an *in vivo* animal model system. The use of QLV modeling to help characterize a multi-component, muscle–tendon system *in vivo* was chosen because of the clinical relevance for tendon repair surgery. Since the tendon of the supraspinatus is short relative to the length of the muscle, *in vivo* biomechanical testing followed by QLV analysis can be used to characterize neuronal influences on the muscle, as the tendon contributions to passive biomechanical properties will likely be negligible in this particular anatomic location.[46] The study hypothesis is that the nervous system contributes to *in vivo* soft-tissue biomechanical properties; therefore, muscle–tendon unit tone, specifically stiffness, and elasticity, can be pharmacologically modulated with BoNT-A.

MATERIALS AND METHODS

The rat shoulder model has been established in previous experiments as a suitable model for the study of human rotator cuff pathology.[42,43] A total of 12 male Sprague-Dawley rats weighing 400–450 g (Charles River, Wilmington, MA) were studied. While housed in the controlled temperature vivarium with 12 h light–dark cycles, chow and water were provided to the rats *ad libitum*. The animals were divided into two experimental groups based upon injection type. Six rats were injected with normal saline and served as controls; the remaining six rats were injected with equal volumes of BoNT-A. The injection protocol is described in greater detail below. At 1 week post-injection, *in vivo* muscle function and biomechanical load-relaxation testing was performed. The animals were killed immediately after *in vivo* testing. Experimentation was blinded in that one scientist injected the animal, and a second scientist performed all surgery and *in vivo* testing. The second scientist was not made aware of the group designation of the animals until after all data was collected and analyzed. All experimentation was conducted after approval from the Institutional Animal Care and Use Committee.

BoNT-A and Saline Injection Procedure

BoNT-A (Allergan, Irvine, CA) was reconstituted as previously described.[26,41,44] Briefly, a 100 U vial of lyophylized BoNT-A was reconstituted with normal saline. The toxin was injected into the supraspinatus muscle at a dosage of 9 units/kg body weight in an injection volume of 10 μL with a Hamilton syringe (Fischer Scientific, Pittsburg, PA) equipped with a 30 gauge needle. The dosage of BoNT-A was determined based on previously published reports in this animal model system and muscle.[5,12] Control animals received an equal volume injection of normal saline (0.9%) in a similar manner.

All injections were performed percutaneously. Animals were anesthetized with isoflurane (Webster Veterinary, Patterson Companies Inc., Sterling, MA) and placed in the prone position. The scapula and shoulder were shaved and prepped with alcohol. The scapular spine was palpated, and the muscle was injected superior and caudal to that bony landmark. The needle was advanced to the bone (suprascapular fossa) and then withdrawn slightly. The injection volume was distributed over the volume of the muscle.

Surgical Exposure and Experimental Apparatus

Saline injected control animals ($n = 6$) and BoNT-A animals ($n = 6$) were anesthetized with isoflurane 1 week post-injection. The time period of 1 week was chosen because the period of time from injection was thought to be sufficient to cause maximal skeletal muscle paresis, which is defined as a reduction in active contractile force. Similarly, we hypothesized that passive biomechanical properties would also change during this initial period of time following injection. Further, we felt that there would be minimal changes in the muscle's structure in the 1 week following BoNT-A injection, making passive biomechanical comparisons of the load–displacement data between experimental groups more likely to be attributable to the neural contributions and less likely to be attributable to structural changes (i.e., atrophy). The injected upper-limbs were shaved and prepped with the animal in the prone position. A skin incision was made over the scapula, extending towards the humerus. The

overlying musculature (deltoid and trapezius) were reflected, and the acromioclavicular ligament was cut, exposing the injected supraspinatus muscle–tendon unit. A custom titanium plate (6.5 mm × 3 mm) with roughened contact surfaces was affixed to the freed supraspinatus tendon. Clamp slippage was not observed during the experimental protocol. A rigidly affixed custom clamp was then securely fastened to a linearly translating experimental table. This custom clamp was attached to the inferior aspect of the scapula, below the scapular spine. The clamp prevented the freely gliding scapula from moving during experimentation. The scapula was gently retracted from the thorax and a brachial plexus dissection was performed to isolate the suprascapular nerve. A bipolar hook electrode was placed on the suprascapular nerve. The nerve plexus and surrounding tissue was electrically isolated by wrapping a piece of Silastic sheeting (Dow Corning Co, Midland, MI) around the exterior of the suprascapular nerve and bipolar electrode. The rat's upper extremity was immobilized with straps and pins on a platform that translated linearly. A single body strap and pins through the wrist and elbow were used to isolate other directional force vectors, ensuring that contractile force and passive force produced by the supraspinatus muscle–tendon unit translated perpendicular and 'in line' with the force transducer in all experimental cases (Fig. 1). If force was not directed perpendicular/'in line' with the force transducer, it is possible that the recorded values of tension would be lower than what is actually produced during active force of contraction and passive stretching protocols.

A steel wire was used to connect the custom titanium plate holding the supraspinatus tendon to a force transducer (model FORT100; World Precision Instruments Inc., Sarasota, FL). The transducer was connected to an amplifier (model 13-G4615-50; Gould, Cleveland, OH) that was interfaced with a personal computer through an interface controller (EMKA TECHNOLOGIES Inc., Falls Church, VA) and an analog-to-digital converter card (model: PCI-6023E, National Instruments, Austin, TX). A pulse generator (model Tenma TGP110, BioSurplus Inc., San Diego, CA) was used to stimulate the suprascapular nerve. The stimulation protocol and the force recordings were controlled and recorded by IOX software, Slow Wave Analyzer (EMKA Technologies Inc., Falls Church, VA). During all experimental recordings, the body temperature of the animals was monitored and maintained with an external heat lamp.

Muscle Force Testing

Supramaximal stimulation of the suprascapular nerve was produced with 1.5 V. A range of 0.6–2.0 V was tested to ensure that maximal single twitch was attained at 1.5 V of stimulation, and in all cases, this stimulation voltage produced maximal single twitch contraction. The muscle–tendon unit was then pretensioned to 0.1 N of preload tension by moving the platform, based on previously published reports in this animal model system,[14,15] and the suprascapular nerve was stimulated at a frequency of 100, 150, and 200 Hz for 1 s, and maximal amplitude of the force of contraction was recorded. A recovery time of 60 s was allowed between stimuli. This recovery time allowed for full recovery of maximal contractile force. A total of three trials were performed at each stimulation frequency, and the largest tetanic contractile amplitude was recorded for each frequency. The stimulation protocol was performed to record the maximal tetanic contractile force for saline injected controls and BoNT-A injected animals 1 week after injection.

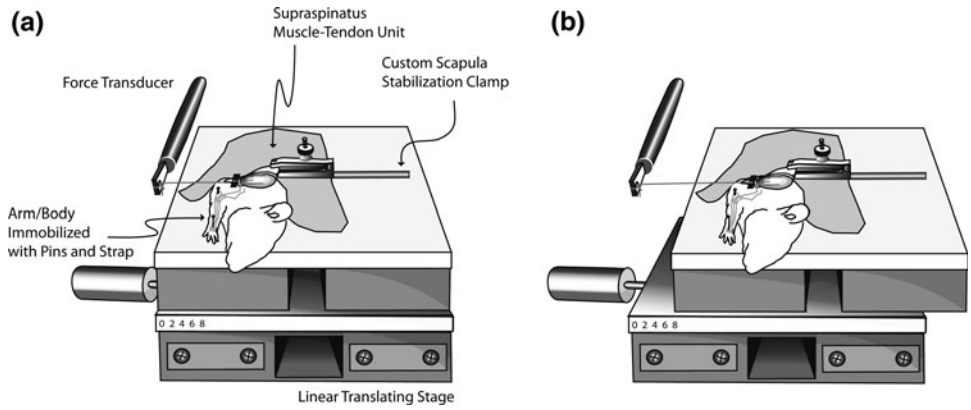

FIGURE 1. Experimental apparatus. The experimental apparatus and key components are shown in this diagram. (a) 0 mm of muscle–tendon unit displacement and (b) 8 mm of muscle–tendon unit displacement (achieved by moving the linearly translating stage away from the force transducer).

Passive Load-Relaxation Testing

A starting preload tension of 0.05 N was set as 0 mm displacement. Prior to the passive load-relaxation testing, a preconditioning protocol of stretching the muscle–tendon unit from 0 to 1 mm was executed and held for a duration of 10 s, then relaxed to 0 mm for 30 s and this was repeated 10 times, based on a previously published report of *in vivo* biomechanical testing in this rat rotator cuff model system.[14] The supraspinatus muscle–tendon unit remained connected to the experimental apparatus after muscle force testing. The stage was then displaced away from the force transducer to specific displacement increments (between 0 and 8 mm). The passive tension of the muscle–tendon unit was assessed *in vivo* by measuring the passive tension produced by the muscle–tendon unit while the muscle–tendon unit was displaced from 0 to 8 mm of displacement in 2 mm increments. Between load-relaxation cycles, the muscle–tendon unit was returned to 0 mm displacement and allowed to rest for 10 min. The passive tension was continuously recorded for a duration of 210 s after displacement, throughout the experimental protocol.

Fung's QLV Model

Fung's QLV model[10] has been used by others to provide a mathematical description of the passive biomechanical behavior of the entire, intact, *in vivo* muscle–tendon unit during loading and relaxation.[12,14] Specifically, the QLV model allows for the extraction of elastic and viscous parameters from the load-relaxation experiments.

Fung[10] proposed that the uniaxial deformation, $\varepsilon(t)$, of a viscoelastic tissue and the resultant uniaxial stress, $\sigma(t)$, exhibited the following relationship:

$$\sigma(t) = \int_0^t G(t-\tau)\frac{\mathrm{d}\sigma^{\mathrm{e}}}{\mathrm{d}t}\mathrm{d}\tau \tag{1}$$

where $G(t)$ is the reduced relaxation function proposed by Fung,

$$G(t) = \frac{1 + C\left(E_1\left(\frac{t}{\tau_2}\right) - E_1\left(\frac{t}{\tau_1}\right)\right)}{1 + C\ln\frac{\tau_2}{\tau_1}} \tag{2}$$

where E_1 denotes the Euler integral $E_1(y) = \int_y^\infty \frac{\mathrm{e}^{-z}}{z}\mathrm{d}z$; $\sigma^{\mathrm{e}}(\varepsilon_{\mathrm{A}})$ is the elastic stress response, and ε_{A} is the applied step increase in strain. In the special nonlinear elastic case,

$$\sigma^{\mathrm{e}}(\varepsilon_{\mathrm{A}}) = A(\mathrm{e}^{B\varepsilon(t)} - 1) \tag{3}$$

where 'A' represents the linear component of the elastic response and 'B' represents the nonlinear component of the elastic response. In our analysis, we assumed the idealized case of the relaxation experiment where an immediate and constant deformation is applied to the tissue such that $\varepsilon(t) = a$. Thus, (1) reduces to

$$\sigma(t) = \frac{A(\mathrm{e}^{Ba} - 1)\left[1 + C\left(-E_1\left(\frac{t}{\tau_1}\right) + E_1\left(\frac{t}{\tau_2}\right)\right)\right]}{1 + C\ln\frac{\tau_2}{\tau_1}} \tag{4}$$

as demonstrated by Nigul and Nigul,[31] where 'C' describes the scaling of the viscous properties during load-relaxation; 'τ_1' describes the early or fast component of relaxation—on the order of seconds; and 'τ_2' describes the late or slow component of relaxation—on the order of minutes to hours. Equation (4) was employed to fit the experimental data using the *nlinfit* nonlinear least squares regression function intrinsic to MATLAB (MathWorks, Natick, MA). Additionally, the goodness-of-fit was quantified for elastic and viscous responses of the QLV model. A coefficient of determination 'R^2' was calculated and used to quantify goodness-of-fit for the nonlinear least squares regression. Others have established this technique as an appropriate and validated method in the rat rotator cuff animal model.[12,14]

When the QLV parameters A and B are varied, the passive tension vs. time curve undergoes a shift and there is a corresponding change in the peak and equilibrium passive tensions (Fig. 2a). Varying parameter 'A' results in a change in the passive tension vs. time graph which scales "linearly" (Fig. 2a); whereas varying parameter 'B' results in a change in the passive tension vs. time graph which scales nonlinearly (Fig. 2a). Varying the QLV parameters 'C,' 'τ_1,' and 'τ_2,' affects the rate of load-relaxation and may influence the equilibrium passive tension (Fig. 2b). Examples of the time histories for passive tension at varying displacements are shown (Fig. 3). These curves (Fig. 3) are parameterized by the Fung's QLV method described previously.

Statistics

All values were expressed as the mean ± standard error of the mean. Statistical analysis was performed using SigmaStat (Systat Software Inc., San Jose, CA). A mixed model ANOVA followed by a Tukey *post hoc* test was performed for between and within group comparison of muscle force measurements stimulated at various frequencies (Fig. 4) and passive tension vs. muscle–tendon unit displacement data (Fig. 5). T tests were performed for comparison of peak stiffness, equilibrium stiffness, and QLV parameters between BoNT-A experimental and saline injected control groups. *In vivo* stiffness of the muscle–tendon unit was determined based on the linear regression analysis (slope of the line) for the tension (peak and

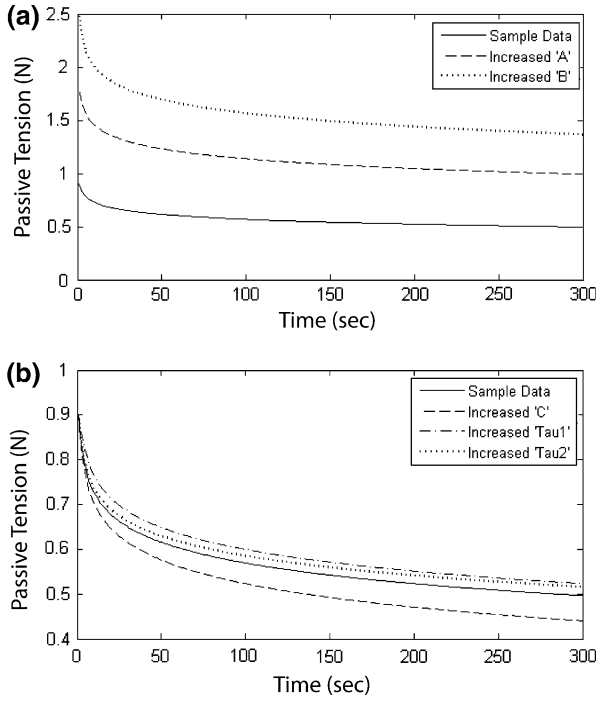

FIGURE 2. The effect of varying QLV model parameters on a sample time history of data for a 4 mm displacement of the rat supraspintus muscle–tendon unit. (a) Demonstrates how doubling parameters '*A*' and '*B*' influence the passive tension vs. time sample data. (b) Demonstrates how doubling parameters '*C*,' 'τ_1,' and 'τ_2,' influence the passive tension vs. time sample data.

equilibrium) vs. displacement data (Fig. 5). Linear regression analysis was performed on the data recorded between 2 and 8 mm on the passive tension vs. displacement data (Fig. 5). At 2 mm of displacement, the preload tension has been documented in the literature[14] to be on the linear portion of the load–displacement curve. Therefore, regression analysis on this portion of the data would presumably avoid the non-linear toe-region.

RESULTS

One week after the injection of BoNT-A, a significant decline ($p = 0.001$) in maximal tetanic contraction force was documented at all frequencies of stimulation when compared to saline injected controls (Fig. 4). At 1 week after the injection, the BoNT-A injected group had an average maximal tetanic contractile force of 0.24 N ± 0.05 N, while the saline injected group had an average maximal tetanic contractile force of 5.06 N ± 0.05 N.

Sample time histories for the control vs. BoNT-A passive force development at various displacements are shown (Fig. 3). BoNT-A significantly reduced the peak passive tension of the muscle–tendon unit as a function of displacement 1 week after injection ($p = 0.022$; Fig. 5a). *Post hoc* analysis revealed that there were significant differences in peak passive tension between experimental groups at 6 mm displacement ($p = 0.002$; Fig. 5a) and at 8 mm displacement ($p = 0.012$; Fig. 5a). Similarly, BoNT-A also reduced the equilibrium passive tension of the muscle–tendon unit as a function of displacement 1 week after injection ($p = 0.043$; Fig. 5b). *Post hoc* analysis revealed that there were significant differences in equilibrium passive tension between experimental groups at 6 mm displacement ($p = 0.013$; Fig. 5b) and at 8 mm displacement ($p = 0.006$; Fig. 5b).

As the displacement of the muscle–tendon unit increased, the passive tensions (both peak and equilibrium) increased linearly (Fig. 5; Table 1). The slopes of the lines representing *in vivo* stiffness of the muscle–tendon unit (Fig. 5) were significantly different between the BoNT-A group and the saline injected group for peak tension ($p = 0.043$; Table 1), as there was a 16.32% reduction in the *in vivo* stiffness of the muscle–tendon unit for the BoNT-A injected group (Table 1). However, a significant difference was not detected for the equilibrium tension ($p = 0.067$; Table 1), as *in vivo* stiffness was calculated from the displacement vs. equilibrium tension data (Fig. 5b), which demonstrated a 22.7% reduction in the stiffness for the chemically denervated experimental group.

QLV parameter A (representing linear scaling of the elastic response) was significantly influenced by chemical denervation with BoNT-A 1 week post-injection (Table 2). No significant differences in the elastic parameter B and the viscous parameters C, τ_1, and τ_2 were detected between experimental groups (Table 2). A nonlinear least squares regression (R^2) was calculated to quantify the goodness-of-fit for all of the time history recordings to the QLV model: average saline $R^2_{\text{elastic}} = 0.9704$ (saline R^2_{elastic} range: 0.8769–0.9997); average saline $R^2_{\text{viscous}} = 0.9968$ (saline R^2_{viscous} range: 0.9888–0.9998); BoNT-A $R^2_{\text{elastic}} = 0.9889$ (BoNT-A R^2_{elastic} range: 0.9757–0.9951); and BoNT-A $R^2_{\text{viscous}} = 0.9990$ (BoNT-A R^2_{viscous} range: 0.9966–0.9997).

DISCUSSION

Neural tone significantly contributes to the passive, *in vivo*, soft-tissue, load-relaxation biomechanical properties of the muscle–tendon unit. Chemical denervation with BoNT-A influences both the active contractile force and passive viscoelastic properties of the muscle–tendon unit. More specifically, the nervous system influences the elastic component of the load-relaxation properties. These findings help to quantify the neural contribution to *in vivo* muscle tone, which is

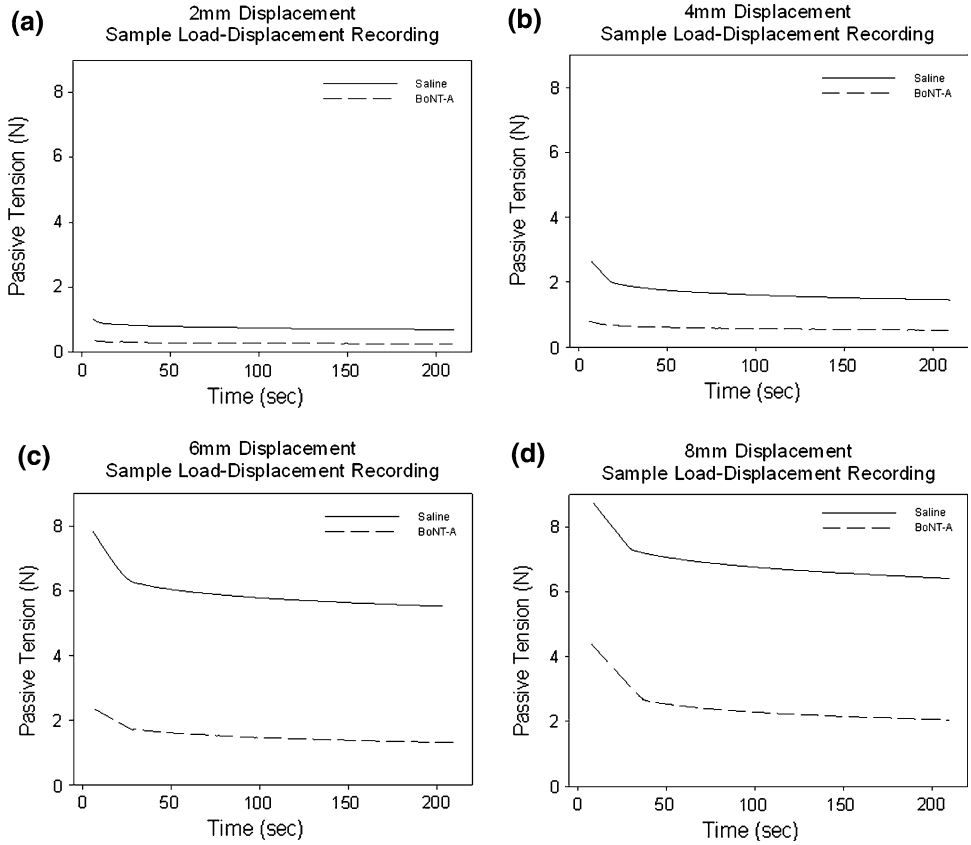

FIGURE 3. Sample time histories of BoNT-A vs. saline injected supraspinatus muscle–tendon units after various displacements. The BoNT-A demonstrated lower passive tension when compared to saline injected controls throughout the time history. Goodness-of-fit of the time histories to the QLV model were assessed by nonlinear least squares regression and reported in the results.

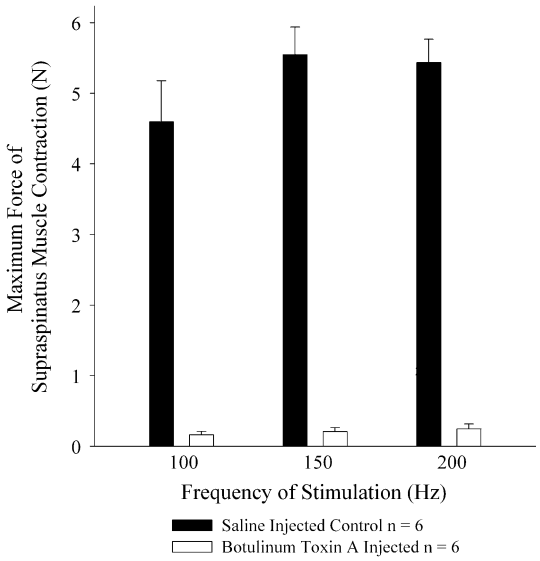

FIGURE 4. The effect of BoNT-A on rat supraspinatus muscle contraction. There is a significant reduction in the maximal tetanic muscle contractile force at all frequencies of stimulation studied at 1 week after injection of BoNT-A when compared to saline injected controls ($p = 0.001$). Data are presented as the mean ± standard error of the mean.

experienced clinically during physical therapy and rehabilitation of patients with insults to their nervous system (i.e. when stretching a spastic muscle of a patient with cerebral palsy, the therapist often experiences a stiff muscle that resists manipulation).[3,22,36,40] Further, pre-operative injection of BoNT-A facilitates the intra-operative surgical manipulation of the muscle–tendon unit by decreasing stiffness (Table 1) and the elastic component '*A*' of Fung's QLV (Table 2). Pre-surgical chemical denervation is a novel approach to modulating the *in vivo* soft-tissue biomechanics of the muscle–tendon unit and may facilitate the mobilization of scarred, retracted, shortened, and atrophied muscle–tendon units during surgery.

Resting muscle tone is attributed to a neural reflex loop, in which afferent sensory nerves detect the contractile state of the muscle and then send a signal to efferent motor neurons, via a synapse in the spinal cord. These efferent motor neurons provide stimulation or inhibition of the muscle, resulting in appropriate contraction or relaxation. In addition to the structural elements (i.e., actin, myosin, titin) of the musculoskeletal system that contribute to passive

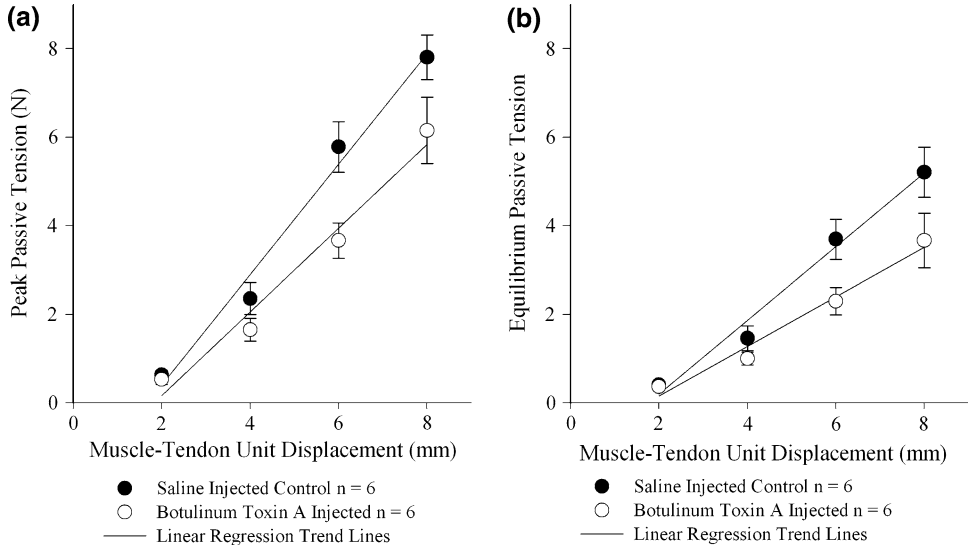

FIGURE 5. The effect of displacement of the rat supraspinatus muscle–tendon unit on passive peak and equilibrium tension. The passive peak and equilibrium tension of the muscle–tendon unit increased as displacement increased. There was a significant difference between the muscle–tendon unit peak passive tension in the BoNT-A injected experimental group compared to the saline injected controls at 1 week post-injection ($p = 0.022$; a). Significant differences in passive equilibrium tension were also detected between the experimental groups at 1 week post-injection ($p = 0.043$; b). The linear regression trend lines are shown for all experimental groups and the data for this analysis are presented in Table 1. Data are presented as the mean ± standard error of the mean.

TABLE 1. Linear regression analysis for rat supraspinatus muscle tendon unit before and after botulinum neurotoxin A injection.

Subject	*n*	Slope stiffness (N/mm)	R^2	*p*
Displacement vs. peak tension (Fig. 5a)				
Average control	6	0.98 ± 0.074	0.952	0.005
Average botulinum toxin A	6	0.82 ± 0.146*	0.934	0.007
Displacement vs. equilibrium tension (Fig. 5b)				
Average control	6	0.651 ± 0.0779	0.947	0.005
Average botulinum toxin A	6	0.503 ± 0.0751	0.94	0.006

Average slope data expressed as Mean ± SEM.
*t test reveals significant difference ($p = 0.040$).

TABLE 2. QLV parameters for rat supraspinatus muscle tendon unit after botulinum neurotoxin A injection.

Subject	*n*	*A* (N)	*B* (mm^{-1})	*C*	τ_1 (s)	τ_2 (s)
Average control	6	2.008 ± 0.996	0.282 ± 0.0573	0.210 ± 0.0382	1.140 ± 0.239	35336.2 ± 4934.438
Average botulinum toxin A	6	0.398 ± 0.124*	0.405 ± 0.0366	0.182 ± 0.0285	1.257 ± 0.251	49718.2 ± 12944.25

Parameters obtained from load-relaxation behavior; all data expressed as Mean ± SEM.
*t test reveals significant difference ($p = 0.02$).

muscle–tendon unit properties, this reflex arc helps to maintain a basal level of contractile tone. The reflex loop is modulated by central nervous system input via the corticospinal and reticulospinal tracts, both of which provide inhibitory modulation of this reflex. BoNT-A blocks the transmission of the efferent nerve input to the skeletal muscle, and thus disrupts the reflex loop.[23] It is likely that the BoNT-A influences elasticity (or QLV parameter '*A*') by disrupting this neural reflex loop, blocking neural input to the skeletal muscle, and reducing stiffness.

Based upon our clinical experience, the effects of a one-time dose of BoNT-A on inhibition of active contractile force are reversed by 4–6 months.[40] Yet in animal studies, modulation of the passive stiffness of the muscle–tendon unit, by a one-time dose of BoNT-A, seem to reverse 2 weeks after injection.[28] Our data demonstrate that BoNT-A significantly

reduces supramaximal contractile force (Fig. 4). Recently, BoNT-A chemical denervation was employed in the perioperative setting, in an animal model, to protect a surgically repaired tendon injury. In this study, the chemically denervated muscles could not generate enough strength to produce suture rupture.[26] Similar favorable outcomes were demonstrated in a clinical tendon repair study.[8] In the non-compliant patient, who attempts excessive activity before healing is allowed to occur following surgery, pre-surgical BoNT-A injection provides "bio-protection" or "chemo-protection" of the repair site because the chemically denervated muscle cannot generate the necessary force to exceed the suture fixation techniques used to secure the torn muscle–tendon unit to the bone.[8,26]

In addition to the proposed "chemo-protection" or "bio-protection" mechanisms, it is likely that repair tension was also influenced by the use of BoNT-A. The healing of a muscle–tendon unit to bone is based on many factors; however, the role of repair tension has recently been appreciated. In the clinically relevant rat rotator cuff animal model, repair tension increased with time after injury.[14] The higher tensioned repairs demonstrated impaired healing at the tendon–bone interface as assessed by biomechanical testing, histology, and molecular assays.[15]

Another rat rotator cuff animal study employed BoNT-A in combination with cast-immobilization of the rodent limb in order to reduce repair tension, with the goal of chemically protecting the surgical repair. In this study by Galatz *et al*., the reduced repair tension coupled with a complete absence of mechanical stimulation during healing led to impaired histological and biomechanical tendon-to-bone healing.[12] Hettrich *et al*. also reported mixed biomechanical and histological results of tendon healing after rotator cuff injury and repair in the rat rotator cuff animal model.[19] However, these studies focused on tendon-to-bone healing and did not quantify the passive biomechanical properties of the muscle–tendon unit. Both studies conclude that further investigation is required at earlier and later time points to determine the efficacy and influence of chemodenervation on tendon-to-bone healing.[12,19]

Based upon previous reports in the literature regarding the relationship between repair tension and tendon-to-bone healing, some investigators have attempted to optimize the mechanical loading of the muscle–tendon unit after surgery. These investigators repaired rotator cuff tendons in the rodent model and then studied various rehabilitation protocols after surgery that employed treadmill running and continuous passive motion.[34,35] One study demonstrated that healing was not impaired with motion as assessed biomechanically and histologically; however, the passive range of joint motion might be adversely affected.[34] The other study demonstrated increased scar formation with excessive early motion, which adversely affected tendon healing.[35] Biologically, some motion or tension at the repair sight is necessary to improve healing; however, repair tensions that are too high result in impaired healing.[4,12,15,34,35,37] Ideally, the BoNT-A dose would be optimized for both pharmacokinetics and pharmaco-dynamics in the pre-surgery setting to correspond with the patient's rehabilitation protocol after tendon repair surgery. In this way, pre-surgical injection of BoNT-A has the potential to allow for the pharmacological control of repair site tension throughout the healing process, further improving the mechano-biology of healing during rehabilitation following surgery.[4,12,15,34,35,37]

Stress and strain is typically used for Fung's QLV analysis of biological tissue, such as tendon, ligament, or muscle. However, QLV parameters in the present study were determined based upon loads and displacements because cross-sectional area and length of the muscle–tendon unit are not known during *in vivo* experimentation. This method has the limitation of being highly sensitive to differences in length and size of the samples used. In the animal model system used, there was enough consistency between the experimental subjects to detect significant differences. Additionally, other investigators have successfully employed the method of parameterization of load–displacement data using an *in vivo* rat rotator cuff animal model system.[14] It is possible that after 1 week of BoNT-A injection, the muscles could have undergone an adaptive response, such as atrophy, which might explain the differences in passive properties observed. A limitation of this study is that muscle volume or physiological cross-sectional area (PCSA) was not measured after experimentation to ensure that these structural changes did not occur. Galatz *et al*. reported minimal structural changes at 14 days post-injection in the muscle's architecture, as determined by histological examination of rat supraspinatus injected with 9 units/kg of BoNT-A, the same dose used in our present study. However, Galatz *et al*. did report some structural changes during their analysis.[12] Further, Hettrich *et al*. reported a significant reduction in rat supraspinatus weight at 4 and 8 weeks after injection.[19] Analysis of stress–strain data, as opposed to load-relaxation data, would account for these potential differences in muscle structure after BoNT-A injection. However, at 1 week after BoNT-A injection, we expect minimal atrophic changes to the muscle's structure.

A limitation of the present study is that only a single dose–volume combination of BoNT-A was examined. Future studies are needed to elucidate the time-course of BoNT-A's modulation on the passive, *in vivo*,

biomechanical properties of the muscle–tendon unit. In addition, studying the effects of BoNT-A over a longer period of time would determine if the stiffness and QLV parameters return to baseline. However, it is possible that the passive properties might not return to the pre-injection state. A report in the literature concluded that repeated BoNT-A injections may result in persistent structural changes in skeletal muscle.[9] However, experience with orthopaedic surgical applications has demonstrated that BoNT-A injections are both safe and reversible.[22,25,40]

The present study also has the limitation of being performed in an animal model. Despite the similarities between the rat and human shoulder, anatomical, structural (i.e., muscle fiber composition, muscle size, muscle volume, etc.), and functional differences (i.e., quadra-pedal vs. bi-pedal) exist between the rodent model and humans. Despite the advantages of *in vivo* testing in the rat model, the present experimental apparatus does not allow for replication of the exact joint biomechanics of normal muscle–tendon–bone action because the rotator cuff muscle–tendon unit was configured linearly with the force transducer to ensure accurate recording. A final limitation was that the *in vivo* biomechanical testing was performed under general anesthesia, which may influence resting muscle tone and function. However, identical muscle–tendon unit testing was performed for both experimental groups, BoNT-A injected and saline injected, under the same anesthetic conditions, with the same anesthetic agent and dose.

Recently, there has been an increased interest in understanding the nervous system's role during the pathogenesis and healing of rotator cuff tears.[16,21,27,38] Since the shoulder is a dynamic joint with a complex range of motion, it requires the simultaneous activation and coordination of many nerves, muscles, tendons, and bones to perform a movement. Our data demonstrate that even at "rest," the nervous system has a significant role in passive skeletal muscle tone, which ultimately influences the motion and function of the joint. The findings of the present study demonstrate that the nervous system influences elasticity of the muscle–tendon unit, as QLV model parameter '*A*' is significantly changed after BoNT-A injection. These findings have implications for mathematical modeling of the musculoskeletal system. Based on our QLV analysis, it is likely that varying elasticity of the tissue in a model system would simulate neural influences. More specifically, our data demonstrate that linear scaling of the elastic tissue response would be most appropriate for investigation of neuronal influences using computational techniques. However, these findings should be interpreted with caution, as further investigation using computational analysis techniques and verification with *in vivo* experimentation is necessary to validate this conclusion.

In conclusion, neural tone significantly contributes to muscle–tendon unit passive biomechanical properties, and pre-surgical treatment with BoNT-A may improve the rehabilitation of muscle by altering the passive elastic properties. Accordingly, pharmacological modulation of skeletal muscle stiffness with BoNT-A increases flexibility and may improve function. Chemical denervation with BoNT-A may also improve the manipulation of stiff and difficult to mobilize muscles during surgical procedures. Besides the rotator cuff, these findings have implications for the operative repair and rehabilitation of many muscle–tendon units, such as biceps tendon, triceps tendon, quadriceps tendon, Achilles tendon, and hand flexor tendons.

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